

RAPHE VESTIGES IN "ASTERIONELLA" SPECIES FROM MADAGASCAR: EVIDENCE FOR A POLYPHYLETIC ORIGIN OF THE ARAPHID DIATOMS?

John P. KOCIOLEK¹ and Kristina RHODE^{2,3}

¹ Diatom Collection, California Academy of Sciences,
Golden Gate Park, San Francisco, CA. 94118 USA

² The Evergreen State College, Olympia, WA. 98505 USA

³ Current Address: Center for Great Lakes and Aquatic Sciences,
2200 Bonisteel Boulevard, The University of Michigan,
Ann Arbor, MI 48109, USA

Dedicated to the memory of Pierre Bourrelly

ABSTRACT — Endemic *Asterionella* taxa from Lake Tsimbazaza, Madagascar, were studied from type material with light and scanning electron microscopy and shown to possess short raphe branches; rimoportulae may be present or absent. These taxa appear to be closely related to *Actinella* species also endemic to Lake Tsimbazaza, by virtue of symmetry and raphe features. Together, these "*Asterionella*" and *Actinella* taxa from Madagascar appear to be part of an early lineage within *Actinella*. Phylogenetic analysis suggests there was a progressive reduction in raphe length and structure in this lineage, as well as the development of stellate colonies. The latter feature may have been in response to the planktonic habit these forms now exhibit. Entire loss of the raphe system in other raphe-bearing groups (documented within the raphidiodioids as well as other raphe-bearing lineages) would yield araphid diatoms. Primitive and secondarily-derived origins of the araphid diatoms would make this group a morphological grade rather than a phylogenetic clade. If phylogeny is to be represented in taxonomy, we suggest the araphid diatoms *sensu lato* do not merit taxonomic recognition as a group.

RÉSUMÉ — Des taxons d' *Asterionella* endémiques du lac Tsimbazaza, à Madagascar, ont été étudiés à partir du matériel type, en microscopie optique et à balayage. Il est apparu que leur frustule possédait de courtes ramifications du raphe, des rimoportulae pouvant être présentes ou absentes. Ces taxons sont apparus étroitement apparentés aux espèces d' *Actinella* endémiques du lac Tsimbazaza, en raison de la symétrie et des caractéristiques du raphe. L'ensemble de ces "*Asterionella*" et de ces *Actinella* de Madagascar apparaît comme une partie d'une lignée ancienne au sein du genre *Actinella*. L'analyse phylogénétique suggère qu'une réduction progressive de la longueur et de la structure du raphe, ainsi que le développement de colonies étoilées se sont produits dans cette lignée. Cette dernière caractéristique pourrait constituer une réponse à l'habitat planctonique que ces formes exhibent désormais. La perte totale du système de raphe chez les autres groupes possédant un raphe (étudié au sein des raphidiodées aussi bien que chez les autres lignées possédant un raphe) aurait conduit aux diatomées araphides. Des origines multiples, primitives et secondairement dérivées, des diatomées araphides auraient fait de ce groupe un gradient morphologique plutôt qu'un clade phylogénétique.

Si la systématique se doit d'être phylogénétique, nous suggérons que les diatomées araphides *sensu lato* ■ soient pas reconnues comme un groupe taxinomique. (Traduit par la Rédaction)

KEY WORDS: *Actinella*, araphids, *Asterionella*, diatoms, Madagascar, phylogeny, polyphyly, raphidoids, taxonomy, ultrastructure.

INTRODUCTION

Manguin (*in Bourrelly & Manguin 1949*) described 6 new *Asterionella* taxa endemic to Lake Tsimbazaza, Madagascar. These include:

Asterionella candelabrum Manguin *in Bourrelly & Manguin*

A. candelabrum f. *baculata* Manguin *in Bourrelly & Manguin*

A. madagascariensis Manguin *in Bourrelly & Manguin*

A. madagascariensis var. *madagascariensis* f. *osseiformis* Manguin *in Bourrelly & Manguin*

A. madagascariensis var. *minor* Manguin *in Bourrelly & Manguin*

A. madagascariensis var. *tibiaeformis* Manguin *in Bourrelly & Manguin*

Körner (1970), in his monograph of the genus *Asterionella*, considered Manguin's species of dubious taxonomic placement within the genus. Although unable to examine original material (or, actually, any material) of these taxa, Körner suggested Manguin's taxa were synonyms of other *Asterionella* species (e.g. *A. candelabrum* = *A. ralfsii* var. *americana* Körner) or of other genera (e.g. *A. madagascariensis* var. *tibiaeformis* = *Eunotia zasuminensis* (Cabejszekowna) Körner). Both species were reported from nearby Mauritius by Coste and Ricard (1984).

Manguin's original material from Lake Tsimbazaza has been located in the Laboratoire de Cryptogamie at the Muséum National d'Histoire Naturelle, Paris (PC), and has served as the basis of a previous paper on *Actinella* Lewis species endemic to this lake (Kociolek *et al.*, 1997). In this paper we provide light and scanning electron microscope observations on valve variation and ultrastructure of Manguin's *Asterionella* species from Madagascar.

MATERIALS AND METHODS

For light microscope (LM) observations of valve components, collections were cleaned in 30% H₂O₂ according to the procedure of van der Werff (1955). Cleaned material was washed with distilled water and settled repeatedly, then air dried onto coverglasses. Coverglasses bearing the dried material were mounted onto glass slides with Naphrax. Material used in all observations of taxa from Madagascar is from Lake Tsimbazaza; PC, "leg. Boiteau 2:3 and 2:4S3"; this is the original material used in the treatment by Manguin (*in Bourrelly & Manguin, 1949*). SEM observations were made on cleaned material air-dried onto coverglasses. The coverglasses were mounted onto aluminum stubs and coated with approximately 20 nm of gold-palladium. Coated stubs were viewed on a Hitachi S520 SEM. Striae measurements, as well as terminology of ultrastructural features are in accordance with standards proposed by Anonymous (1975) and Ross *et al.* (1979).

RESULTS

Asterionella candelabrum Manguin in Bourrelly & Manguin 1949, p. 165, pl. 5, fig. 58
Figs 1-14

Light Microscopy. Valves are asymmetrical about the transapical axis, and also slightly asymmetrical about the longitudinal axis (Figs 3-6). The headpole is expanded and the apex has a slight notch. The footpole is narrow and rounded. The middle portion of the valve is expanded slightly. Striae extend across the valve; they are not interrupted by a central sternum. Length, 26-43 μm , breadth 2 μm . Striae 18-26/10 μm .

SEM. External views show some valves may be slightly twisted about the apical axis (Fig. 9). Small spines are present on the margin but more prominent at the footpole. Round puncta extend across the face of the valve and onto the mantle. At the headpole (Fig. 10) and footpole (Fig. 11) a small slit-like opening can be found on the valve mantle of the ventral margin. The opening may extend onto the valve face (Fig. 12). Internally, at the both headpole (Fig. 13) and footpole (Fig. 14), small slit-like openings are found on one side of the valve at or near the face:mantle interface. The slit-like openings lack any additional structure.

Comment. The feature used by Manguin to segregate the taxon *A. candelabrum* f. *baculata*, namely a lack of swelling in the median part of the valve, could not be recognized. Thus we consider this taxon as part of the nominate form.

A. madagascariensis Manguin in Bourrelly & Manguin 1949, p. 165, pl. 5, fig. 60
Figs 15, 16; 21-26

Light Microscopy. Valves are straight to almost C-shaped, bent to almost 90 degrees about the apical axis; asymmetrical about the transapical axis and, to a slight degree, about the apical axis. Headpole swollen, rounded; footpole rounded. Striae appear to extend across the valve face. Length, 50-70 μm , breadth 3 μm . Striae 18-20/10 μm .

SEM. Internal views show striae composed of round puncta that extend across the valve face (Figs 23-25). At the poles striae are arranged in a radiate fashion (Figs 22, 23, 26). A small rimoportula is positioned on the mantle at one end of the valve, and a small slit-like opening may be positioned on the valve face (Fig. 26). The slit-like opening differs from the rimoportula by lacking siliceous swellings on both sides along its longitudinal axis.

Comment. Specimens grade from straight to slightly bent to strongly arched. Valve shape was used Manguin to segregate *A. madagascariensis* var. *madagascariensis* f. *osseiformis* from the nominate. Given the continuum of variation expressed in valve shape, we consider the two taxa as synonyms.

A. madagascariensis var. *minor* Manguin in Bourrelly & Manguin 1949, p. 166, pl. 5, fig. 62
Figs 17-20; 27-30

Light Microscopy. Valves narrow, slightly asymmetrical about the transapical axis, distinctly asymmetrical about the apical axis, headpole bluntly rounded, footpole narrowly rounded. Terminal nodules are often distinct. Striae extend across valve face. Length 21-25 μm , breadth 1.5-2.0 μm , striae 20-24/10 μm .

SEM. Valves are shown to possess small, irregularly-placed spines along the periphery of the valve; spines are more concentrated at the apices than along the rest of the valve (Figs 27, 28). Round puncta comprise the striae, which extend across the valve face (Figs 27-29). Striae also extend onto the valve mantle. Small raphe branches are visible along the ventral margin (Fig. 27), restricted to the mantle except the extremity of the distal raphe end (Fig. 28). Internally the raphe terminates as helictoglossae, and a single, well-developed rimoportula is found on the mantle at one end only (Figs 29, 30).

A. madagascariensis var. *tibiaeformis* Manguin in Bourrelly & Manguin 1949, p. 166, pl. 5, fig. 63, pl. 6, fig. 64

Figs 31-41

Light Microscopy. Valves only slightly asymmetrical about the transapical axis, distinctly asymmetrical about the apical axis. Ends bluntly rounded with a slight swelling at the middle part of the valve. Striae extend across the entire valve face. Length, 20-28 μm , breadth 1.5-2.5 μm . Striae 16-22/10 μm .

SEM. Valves are bent in the apical plane (Fig. 35). Heterovalvy is demonstrated with regard to the development of the raphe branch. On some valves the raphe may be distinct and extend from the mantle onto the face, in other valves the raphe may be small, irregularly arranged and restricted to the mantle (Figs 37, 38). Girdle bands are numerous and are of the open type. Some are continuous around the headpole and others around the footpole. Bands bear 1-3 rows of poroids; the poroids are of the same size and shape as puncta (Fig. 38). The valve face is bordered by small conical spines (Figs 36, 37). Internally, striae appear interrupted near the ventral margin, but continue onto the mantle. The raphe terminates as helictoglossae and at the footpole there is a single rimoportula (Figs 40, 41).

DISCUSSION

Considering the suite of features observed in the *Asterionella* taxa described by Manguin, including: a) presence of a raphe system, b) raphe system short, restricted to the mantle or just arching onto the valve face at the distal raphe ends and c) asymmetry about both the apical and transapical axes, it seems appropriate to assign these taxa to the genus *Actinella*. We therefore propose the following new combinations:

***Actinella candelabrum* (Manguin in Bourrelly & Manguin) Kociolek & Rhode, comb. nov.**

Basionym: *Asterionella candelabrum* Manguin in Bourrelly & Manguin 1949, p. 165, pl. 5, fig. 58. Contribution à l'étude de la flore algale d'eau douce de Madagascar: Le Lac Tsimbazaza. *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 161-190 + pls 1-7.

Synonym: *A. candelabrum* f. *baculata* Manguin in Bourrelly & Manguin 1949, p. 165, pl. 5, fig. 59.

***Actinella madagascariensis* (Manguin in Bourrelly & Manguin) Kociolek & Rhode, comb. nov.**

Basionym: *Asterionella madagascariensis* Manguin in Bourrelly & Manguin 1949, p. 165, pl. 5, fig. 60. Contribution à l'étude de la flore algale d'eau douce de Madagascar: Le Lac Tsimbazaza. *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 161-190 + pls 1-7.

Synonym: *Asterionella madagascariensis* var. *osseiformis* Manguin in Bourrelly & Manguin 1949, p. 165, pl. 5, fig. 61.

***Actinella bourrellyi* (Manguin in Bourrelly & Manguin) Kociolek & Rhode, comb. nov.**

Basionym: *Asterionella madagascariensis* var. *minor* Manguin in Bourrelly & Manguin 1949, p. 166, pl. 5, fig. 62. Contribution à l'étude de la flore algale d'eau douce de Madagascar: Le Lac Tsimbazaza. *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 161-190 + pls 1-7.

***Actinella reviersii* (Manguin in Bourrelly & Manguin) Kociolek & Rhode, comb. nov.**

Basionym: *Asterionella madagascariensis* var. *tibiaeformis* Manguin in Bourrelly & Manguin 1949, p. 166, pl. 5, fig. 63, pl. 6, fig. 64. Contribution à l'étude de la flore algale d'eau douce de Madagascar: Le Lac Tsimbazaza. *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 161-190 + pls 1-7.

Within *Actinella*, these species appear closely related to other endemic *Actinella* from the same lake (Fig. 42). Kociolek *et al.* (in press) showed that the Lake Tsimbazaza taxa are primitive within *Actinella*, all lacking an apical point at the headpole. Amongst these primitive members of *Actinella*, it would appear that raphe reduction, including reduction in size and reduction/loss of helictoglossa is a derived feature. Many examples exist elsewhere of the secondary reduction/loss of raphe systems, including that for monoraphid diatoms (e.g. Andrews, 1981), *Navicula* Bory (Hustedt, 1962; Lange-Bertalot & Le Cohu, 1985) and raphidoid taxa such as *Peronia* Brébisson & Arnott *ex* Kitton (Gemeinhardt, 1926) and *Eunotia* Ehrenberg (Hustedt, 1952). Reduction of the raphe system in *Actinella* species might be related to its evolution from a benthic to a planktonic life form strategy. Species with more typical raphidoid raphe systems exhibit zig-zag colonies, similar to other *Actinella* species (e.g. *A. punctata* Lewis) while those with reduced raphe systems have stellate colonies (Manguin in Bourrelly & Manguin, 1949). A proposed set of relationships within this set of *Actinella* species is presented in Fig. 43.

If we can envision a total loss of the raphe system in *Actinella*, as suggested to have happened within *Eunotia* several times (Hustedt, 1952) and in *Navicula* (Hustedt, 1962), this would be additional evidence to suggest a polyphyletic origin of the araphid condition. Such a view opposes the traditional systematic placement of the araphids as a primitive group from which the ur-raphid diatom was to emerge. Proponents of such a view included Berg (1948) and Kolbe (1956), and it is inherent in the classification scheme

of Round *et al.* (1990) where the araphids are recognized as a separate class (similar to the scheme proposed by H.L. Smith, 1872). Primitive and secondarily-derived origins of the araphid diatoms would make this group a morphological grade rather than a phylogenetic clade. A polyphyletic origin of the araphids has received recent support from molecular data, where Sorhannus *et al.* (1995) showed that some araphids may be primitively so, while others, including "*Asterionella*" may be more derived.

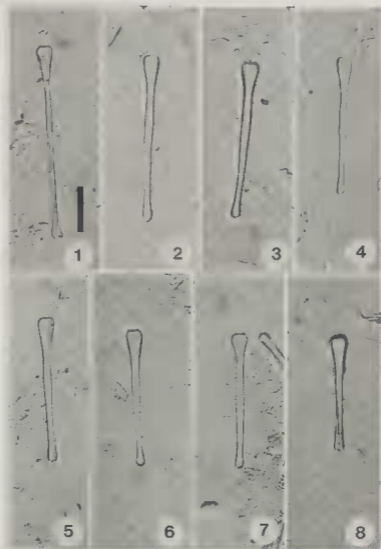
Our classification systems should reflect the evolutionary relationships of the organisms they represent. The current system of recognizing a separate class for the araphid diatoms, given the evidence across many groups for their polyphyletic origin, would appear to be in conflict with this goal for classifications. We advocate recognition of only monophyletic groups in classification schemes, and therefore suggest no formal taxonomic designation for the araphids *sensu lato*.

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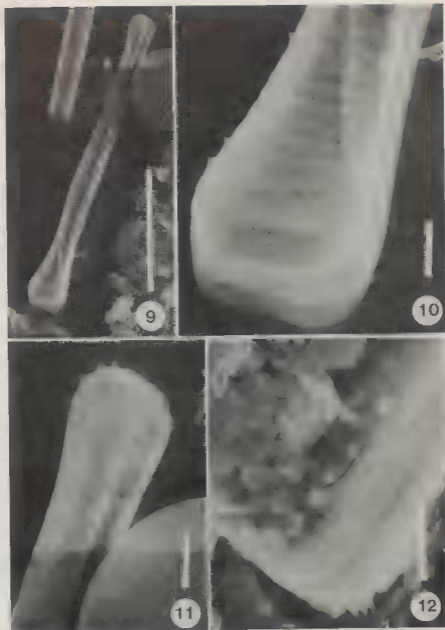
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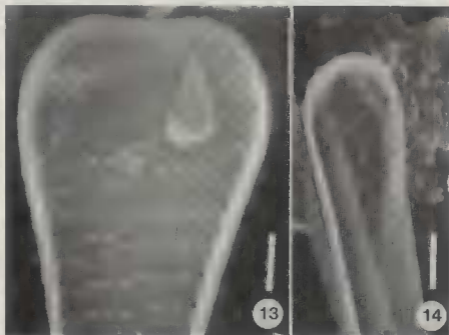
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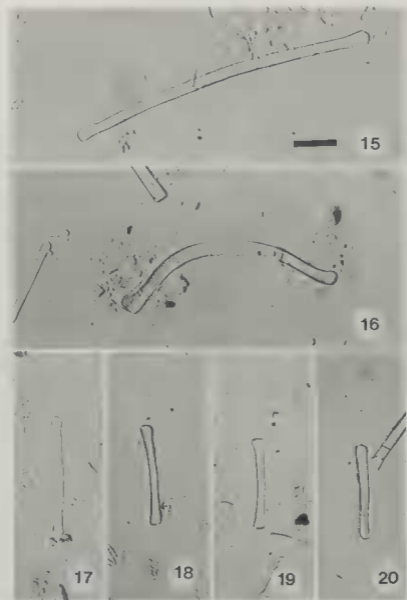
Figs 1-8. *Actinella candelabrum*, L.M. Valve views. Scale bar = 10 μ m.



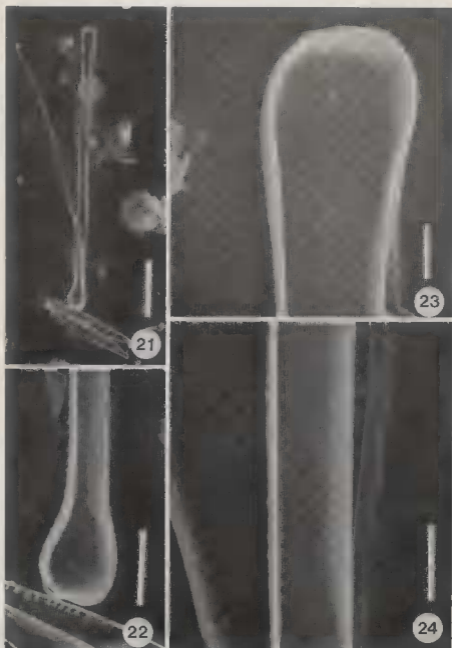
Figs 9-12. *Actinella candelabrum*. SEM. External valve views. Fig. 9. Valve view showing slight twist to valve, where the footpole is twisted out of the plane of the headpole. Scale bar = 10 μ m. Fig. 10. Headpole of specimen in Fig. 9. Valve face is slightly concave, no prominent central sternum is present; a few small spines are located around the valve periphery. A small, irregular opening is present at the valve face:mantle junction. Scale bar = 1 μ m. Fig. 11. Valve view of footpole of specimen in Figs 9-10, showing larger spines at valve terminus and small opening on valve face:mantle junction. Opening at footpole is on same side of valve as at headpole. Scale bar = 1 μ m. Fig. 12. Girdle view of footpole showing opening extending from valve mantle onto face. Round pores and small spines are also visible. Scale bar = 1.25 μ m.



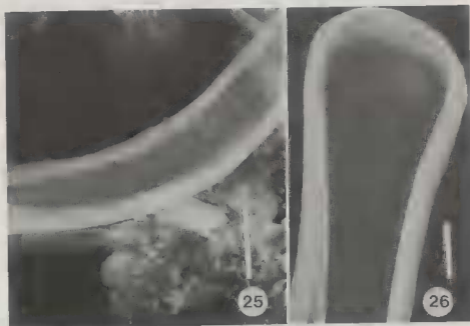
Figs 13-14. *A. candelabrum*. SEM. Internal views. Fig. 13. Headpole showing slit-like opening without helictoglossa and rimoportulae. Lack of central sternum is demonstrated. Scale bar = 1 μ m. Fig. 14. Footpole showing small slit-like opening and possible rimoportula (arrow). Scale bar = 1.25 μ m.



Figs 15-20. LM. Valve views. Figs 15, 16. *Actinella madagascariensis*. Figs 17-20. *Actinella bourrellyi*. Scale bar = 10 μ m.



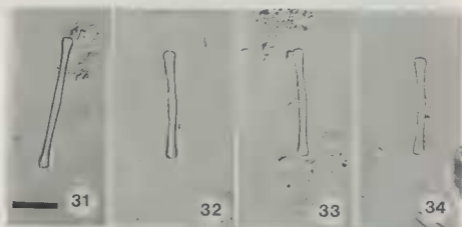
(Legends to Figs 21-24 are on p. 69).



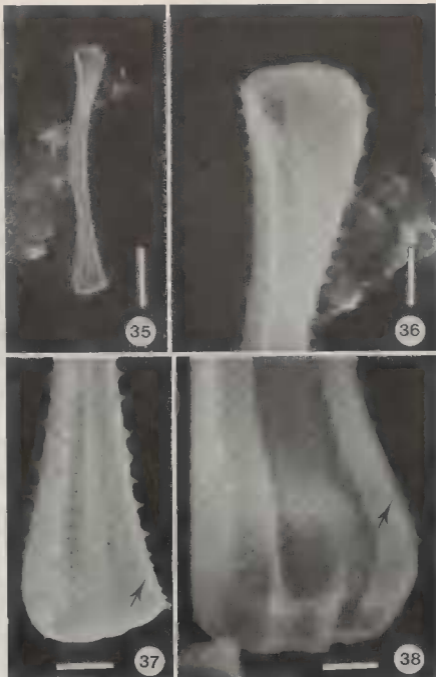
Figs 21-26. *Actinella madagascariensis*. SEM. Internal views. Fig. 21. Valve view showing overall outline of straight specimen. Scale bar = 10 μm . Fig. 22. Headpole of specimen in Fig. 21 showing shape of headpole. Scale bar = 4 μm . Fig. 23. Median part of valve from specimen in Fig. 21, showing striae traversing valve face. Scale bar = 1.25 μm . Fig. 24. Footpole showing striae radiating around pole and presence of rimoportula. Scale bar = 2 μm . Fig. 25. Central portion of curved specimen showing striae extending across valve face. Scale bar = 3 μm . Fig. 26. Footpole showing curved radiate nature of striae at the pole and presence of small, slit-like opening. Scale bar = 1.25 μm .



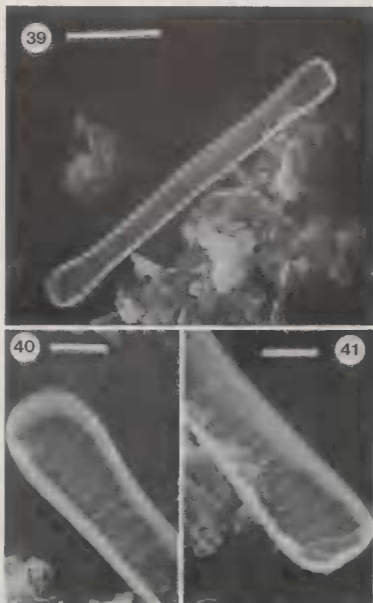
Figs 27-30. *Actinella bourrellyi*. SEM. Figs 27, 28, External views. Fig. 27. Valve view showing general valve outline, spines irregularly scattered around the periphery of the valve and distinct raphe branches at both ends of the ventral margin. Scale bar = 5 μ m. Fig. 28. Footpole of specimen shown in Fig. 27, with details of the striae, small spines and raphe branch restricted mostly to the mantle but with distal raphe end curved onto the valve face. Scale bar = 1 μ m. Figs 29, 30, Internal views. Fig. 29. Headpole showing raphe with small helictoglossa. Striae are shown to extend onto valve mantle. Scale bar = 1 μ m. Fig. 30. Footpole with raphe ending in distinct helictoglossa and mantle bearing rimoportula. Scale bar = 1 μ m.



Figs 31-34. *Actinella reyersii*, LM. Valve views. Scale bar = 10 μm .



Figs 35-38. *Actinella reyersii*. SEM. External views. Fig. 35. Girdle view showing frustule bent about apical plane. Valve is bordered by small conical spines. Scale bar = 5 μm . Fig. 36. Footpole of specimen illustrated in Fig. 35. Conical spines are shown, and several girdle bands are evident. Small raphe branches restricted to the valve mantle are evident on both valves. Scale bar = 1.25 μm . Fig. 37. Headpole of specimen shown in Figs 35, 36. One valve has raphe branch that extends from mantle to valve face while the second valve has small, irregular raphe branch that is restricted to valve mantle. Scale bar = 1.25 μm . Fig. 38. Another example of heterovalvy with respect to raphe branches: one is arched from mantle to face, the other is small, irregular and restricted to mantle. Large valvocopula has three rows of poroids. Scale bar = 1 μm .



Figs 39-41. *A. reversii*. SEM. Internal views. Fig. 39. Valve view showing organization of striae. Scale bar = 5 μ m. Fig. 40. Same specimen as Fig. 39, headpole with raphe ending in helictoglossa. Scale bar = 1.25 μ m. Fig. 41. Footpole of same specimen illustrated in Figs 39, 40 showing raphe ending in helictoglossa and possible rimoportula (arrow) positioned on mantle. Scale bar = 1.25 μ m.

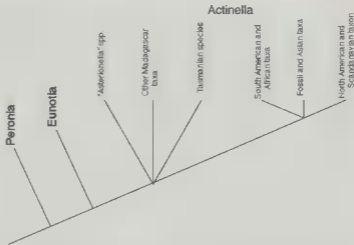


Fig. 42. Interrelationships of *Actinella* species groups. See text for details.

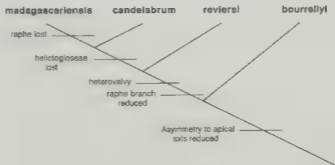


Fig. 43. Proposed set of interrelationships of *Actinella* species previously identified as *Asterionella* from Lake Tsimbazaza, Madagascar.